THE EFFECT OF 2,4,5-TRICHLOROPHENOXYACETIC ACID ON THE DISTRIBUTION OF AN ARTIFICIAL AMINO ACID IN SELECTED TISSUES OF PREGNANT RATS

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SUMMARY

A study was undertaken to provide information about a possible mode of fetotoxic action of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in rats.

On day 14 of gestation, rats were given a dose of 150 mg/kg 2,4,5-T in mineral oil via oral intubation. Four hours later, each animal was injected interperitoneally with the artificial amino acid, α-aminoisobutyric-1-C^{14} acid. Ninety minutes after injection, rats were sacrificed and the distribution of the amino acid in maternal plasma and liver and whole embryo was compared against controls which did not receive 2,4,5-T.

Other groups of rats were fed 50 or 100 mg/kg 2,4,5-T on days 9 through 14 of gestation. On day 20 embryos were removed, weighed and compared to the embryos of pair-fed or untreated controls.

CHAPTER I

INTRODUCTION

The herbicide, 2,4,5-trichlorophenoxyacetic acid (Figure 1), had been the subject of considerable teratological investigation for the past seven years. The interest in this compound, commonly called 2,4,5-T, grew in large part out of work done at the Bionetics Research Laboratories in Bethesda, Maryland, in the late 1960's (Courtney, et al., 1970). The report attributed teratogenic potential to 2,4,5-T but also revealed that the sample of the herbicide tested contained significant amounts of a highly toxic contaminant. Subsequently, research was generated in several laboratories to test the validity of the original findings. In addition, the distribution of the compound in experimental animals was investigated. However, little, if any, effort has been made to determine the biological activity of this herbicide in animals which are subject to its reported developmental effects. The purpose of this study is to develop and test an hypothesis which will provide information in this area.

Courtney, et al. (1970) reported that 2,4,5-T was teratogenic when administered to mice in doses ranging from 21.5 to 113 mg/kg/day for eight or nine days during gestation, 2,4,5-T was teratogenic. The route of administration, subcutaneous injection or oral, had no effect on the results. The most pronounced effects included high incidences of cleft palate and cystic kidney. In doses ranging from 4.6 to

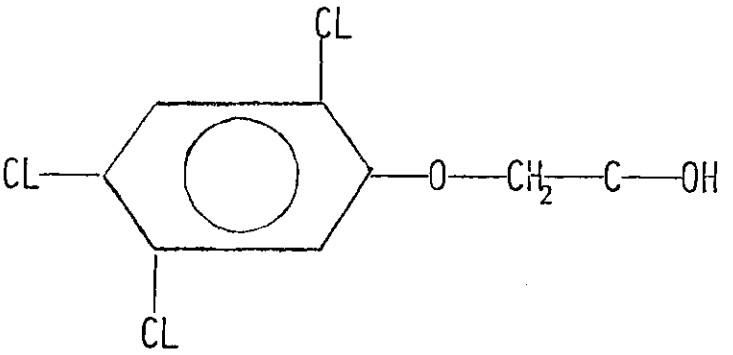

Figure 1. 2,4,5-Trichlorophenoxyacetic Acid

46.4 mg/kg/day on days 10 through 15 of gestation, 2,4,5-T was fetotoxic and teratogenic in rats. The sample of the herbicide used contained 30 ppm 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), which is a highly toxic by-product of 2,4,5-T synthesis. Its presence in the 2,4,5-T tested rendered the results of the study suspect, at best.

Courtney and Moore (1971), in a second investigation of the developmental effects of 2,4,5-T, used a formulation of the compound which was essentially free of TCDD. Pregnant rats were treated with doses of 10 to 80 mg/kg/day, and pregnant mice received doses 50 to 150 mg/kg/day. Fetal mortality was increased in both animals but only at the highest doses. Fetal weight was reduced in the mouse at doses greater than 100 mg/kg/day but not at any dose in the rat. There was a depression of maternal weight gain in one strain of mice and at the higher doses in rats. The terata found in the earlier study, namely cleft palate and cystic kidney, were found only in mice.

Dow Chemical Company is one of the primary producers of 2,4,5-T and maintains laboratories to determine the hazards involved in the manufacture and use of such compounds. These laboratories have been the source of several reports dealing with the effects of 2,4,5-T on development. Emerson, et al. (1971), found that 2,4,5-T containing less than 0.5 ppm of TCDD, when administered to pregnant rats and rabbits during the period of organogenesis, was not teratogenic and did not adversely affect fetal size. The range of doses employed was one to 24 mg/kg/day. Sparschu, et al. (1971), administered 2,4,5-T to rats in doses of 50 or 100 mg/kg/day during days 6 through 15 of

pregnancy. The higher dose was toxic to the females and was terminated after five days. Maternal weight gain was unaffected at the lower dose but was reduced significantly at the higher, toxic dose. Resorption was increased at 100 mg/kg/day and fetal weight was reduced. A significant number of skeletal anomalies were reported at both doses but discounted by the authors.

Khera and McKinley (1972) have shown that rats administered 2,4,5-T on days 6 through 15 of gestation at doses of 100 and 150 mg/kg/day exhibited the following effects: (1) reduced fetal weight; (2) increased number of dead fetuses; and (3) greater proportion of fetuses with skeletal anomalies. Investigation of the embryotoxic effects of 2,4,5-T on mice was carried out by Neubert and Dillman (1972), who found that doses of the pure herbicide ranging from 30 to 120 mg/kg/day caused a significant increase in the incidence of cleft palate and resorptions. These authors found a decrease in fetal and maternal weight, even at the lower end of the dose range.

Before the publication of the Bionetics Laboratories study, Courtney (1970) had already investigated the metabolism and distribution of 2,4,5-T and related compounds in the rat. Excretion in the urine of 90% of the 2,4,5-T injected subcutaneously occurred within 72 hours. Analysis of the urine indicated an excretion rate of 300 ug/hr and that the compound was essentially unmetabolized. Serum levels of the herbicide were dose dependent and peaked 4 to 12 hours after injection. Various pretreatments also affected serum levels. Recovery of 2,4,5-T from 15 day fetuses established that placental

transport did occur and seemed to be dependent on maternal serum levels.

Fang, et al. (1973), used C^{14}-labelled 2,4,5-T to study the distribution and metabolism of 2,4,5-T in the rat. In the 24 hours following administration via stomach tube, as much as 75 to 80% of the compound could be recovered in the urine and feces. Eventually, up to 96% was recovered, indicating very limited metabolism. Distribution was throughout all tissues, the kidney accumulating the highest concentration. It was followed in order of decreasing concentration by the blood, the lungs, the heart and the liver. Maximum levels were reached 6 to 12 hours after feeding. In pregnant animals, the placenta had the highest concentration of any tissue with the exception of the kidney. Transport of the fetus occurred and appeared to be dependent on the flow-rate of maternal blood to the placenta. The half-life of 2,4,5-T in tissue was 3.4 hours in the adult and 97 hours in the neonate.

Lindquist and Ullberg (1971) administered C^{14}-labelled 2,4,5-T to mice and determined by whole body radiography that it accumulated in the yolk sac epithelium. These authors proposed that 2,4,5-T acts by interferring with embryotrophic nutrition, which is carried out by the yolk sac and is important in rodents until day 10 of gestation.

The literature does not offer conclusive evidence of teratogenicity of 2,4,5-T in rats, as it does in mice. Khera and McKinley (1972) and Sparschu, et al. (1971), attributed a reduction of fetal weight in rats to 2,4,5-T, while Neubert and Dillman (1972) reported

similar results in mice, as did Courtney, et al. (1971). These findings on fetal weight reduction are open to question. In the study by Sparschu, et al. (1971), the dose and/or vehicle or combination of the two was quite toxic. The study by Courtney, et al. (1971), utilized 2,4,5-T with high TCDD content. Furthermore, since pair-fed controls were apparently not used in any of the studies mentioned, the obviously important variable of food intake has not been controlled. The studies on 2,4,5-T distribution in the rat and mouse indicate that the placenta accumulates rather high concentrations and forms no barrier against transport to the fetus.

The placenta is the organ which provides for fetal nutrition. Since 2,4,5-T is concentrated by the placenta, it seem reasonable to question that there may be some effect on fetal nutrition in animals given the herbicide on a daily basis during gestation. The structure of 2,4,5-T is similar, with important differences, to that of an aromatic amino acid. A disruption of amino acid transport across the placenta could have the effect of retarding fetal growth, particularly if it occurred over a period of normally active protein synthesis.

Christensen (1965) has made extensive use of artificial amino acids to study amino acid transport. The most often used artificial amino acid is α-aminoisobutyric acid (Figure 2). The advantage in the use of such compounds is that while they are recognized by the transport systems as amino acids, they are not metabolized and their movements and net transport are more easily determined. Feldman and Christensen (1962) have shown that α-aminoisobutyric acid (AIB) is

suitable for the study of amino acid transport across the placenta.

It was determined on the basis of the foregoing facts and reasoning to test the following hypothesis: the herbicide, 2,4,5-T, will influence the transport of AIB across the rat placenta. In addition, it was determined to carry out pair feeding studies to test the hypothesis that 2,4,5-T causes a reduction of fetal weight when administered to rats on days 9 through 14 of pregnancy.

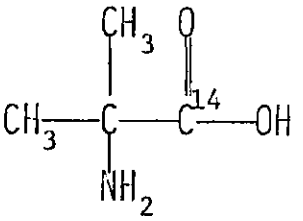

Figure 2. α-aminoisobutyric-1-C^{14} Acid

CHAPTER II

MATERIAL AND METHODS

Rats used in this experiment were of a Sprague-Dawley strain which has been maintained for some years by the School of Biology at the Georgia Institute of Technology. All females resulted from the same breeding and were themselves bred for this study between the ages of 100 and 120 days. Virgin females were placed three to a cage with two males. Day zero of pregnancy was declared when sperm were detected in a vaginal smear.

Animals were maintained on a schedule of 12 hours light and dark, at 70±3°F, with unlimited access to water. Rats were caged individually in plastic cages or groupwise in stainless steel cages, according to experimental regimen. All were maintained on Purina Rat Chow; the amount varied with experimental regimen.

Analytical grade 2,4,5-T was generously furnished by the Dow Chemical Company in the form of a white crystalline powder. New England Nuclear Corporation was the source of α-aminoisobutyric-1-C^{14} acid, specific activity 12.2 millicuries/mM. Common mineral oil was used as a suspension media for 2,4,5-T. Aquasol (New England Nuclear) was the scintillation cocktail. Glacial acetic acid, tricarbosylic acid, sodium fluoride and potassium oxalate were standard commercial preparations, used without further purification.

Two types of rat cages were used. Plastic bottomed cages with

wire tops were from Fisher Scientific. These were equipped one each with 1/2-pint Mason jars suspended from the side of the cage by a stiff wire, for the purpose of containing food and minimizing its spillage. Sani-Cel and Aspen animal litters were used and changed daily. Stainless steel cages were products of the Acme Metal Company and were built with wire mesh floors.

Purina Rat Chow was coarsely ground with an ordinary food grinder to allow more accurate weighing and to prevent the animals from removing more food from the receptacles than they could eat. Food was weighed on a Dietic Model 1440 scale. Rats were weighed on an Ohaus triple beam balance equipped with an animal basket. Embryos and tissues were weighed on a Mettler H-16 balance. Liquid scintillation counting was done on a Mark I Liquid Scintillation Counter, Model Number 6894, made by Nuclear Chicago. Tissue homogenization was carried out in a VirTis Model 23 homogenizer.

The experimental regimen of rats used to test the effect of 2,4,5-T on the distribution of AIB-1-C^{14} acid consisted of food and water ad libitum until day 13 of gestation. In the evening of that day food was removed. On the morning of day 14, each animal was assigned, by coin toss, to one of two groups. Animals in the experimental group were force fed a dose of 2,4,5-T, 150 mg/kg, suspended in mineral oil, 3.35 ml/kg. This is well below the LD_{50} of 300 mg/kg for rats (Herbicides Handbook, 1970). The control group was force fed an equivalent amount of mineral oil. Animals were treated serially beginning at this point. After four hours animals of both groups were injected interperitoneally with AIB-1-C^{14} in saline solution on the

basis of 10 uC/kg body weight, a dose equivalent to 0.84 mg/kg body weight. The total volume of the injection varied from 0.2 to 0.3 ml per animal.

Ninety minutes after it had been injected, one ml of maternal blood was obtained by cardiac puncture and the animal was sacrificed. The sample of blood was combined with a few crystals of the anticoagulants NaF and $K_2C_2O_4$ and reserved for later analysis. Each animal was opened and a slice of maternal liver and the upper three embryos from each horn of the uterus were removed, weighed and placed on ice. The following analysis is that of Feldman and Christensen (1962).

Blood was centrifuged and 0.5 ml of plasma was obtained. To this was added four volumes of 10% TCA solution. The resulting precipitate was removed by centrifugation. Liver and embryo were added to a volume of 0.1 N acetic acid equal to the weight of the tissue sample. After homogenization, a volume of distilled water equal to 10 times the original tissue weight was added. A second homogenization was carried out and the resulting mixture was held in boiling water for three minutes, and the coagulant removed by centrifugation. At this point, 0.2 ml of the supernates were added to 9.8 ml Aquasol in a 15 ml scintillation vial and counted for one minute.

Rats used to test the effect of 2,4,5-T on fetal weight underwent the following treatment. Eight pregnant rats were called Group I. Beginning with day zero of pregnancy, they received food and water ad libitum. The food intake and weight of each animal was measured and recorded daily. Beginning on day 9 and continuing through day 14,

each animal was force-fed a dose of 2,4,5-T suspended in mineral oil on the basis of 100 mg/kg. On day 20 of gestation, animals were sacrificed by .etherization and the embryos counted, removed and individually weighed. Dead fetuses and corpora lutea were also recorded.

Group II was a control group and consisted of eight pregnant animals, each of which was paired by weight, as nearly as possible, with a member of Group I. Each animal in Group II received daily an amount of food equal to the amount eaten by its Group I pair-mate during the same day of gestation. Group II received mineral oil only on days 9 through 14; otherwise, they were treated precisely as Group I.

Group III was eight pregnant animals whose treatment was identical to that of Group I except that the dose of 2,4,5-T was 50 mg/kg. Group IV was a control group related to Group III as Group II was to Group I. Group V was seven pregnant animals who received food ad libitum and mineral oil on days 9 through 14. Group VI was six pregnant animals which were untreated except for daily weighing.

Several animals were not pregnant, despite a positive vaginal smear, in which case their pair-mate was not included in the data. One animal in Group I died during treatment, as did one member of Group IV. Their pair-mates were also excluded from the data. This policy resulted in smaller groups than originally anticipated and smaller than desired for statistical analysis but does represent true pair-feeding. Likewise, in the study of AIB-1-C^{14} acid distribution,

animals which did not yield a full set of data, i.e. plasma, liver and embryo (based on a minimum of three embryos in each horn of the uterus), were not included in the data.

Statistical analysis of the data included acceptance of the null hypothesis unless the level of significance was 5% or better. Means of control and experimental groups were compared by the "Student's" t-Test (Williams, 1968). Since fetuses of a litter tend to resemble each other, it is improper to combine litters within groups for parametric comparison of the groups. Accordingly, Wilcoxon's signed rank test (Sokal and Rohlf, 1973) was employed to compare the mean fetal weights of the litters in one group with those of another. The Mann-Whitman U Test, for non-parametric comparison of unpaired data, was used to test for variation between the control groups (Sokal and Rohlf, 1973).

CHAPTER III

RESULTS

AIB-1-C^{14} Acid Distribution

The data compiled in Tables 1, 2 and 3 represents the counts per minute recorded for the specific tissue samples. Since it has been shown (Feldman and Christensen, 1962) that metabolic transformation of AIB does not occur to any appreciable extent, counts will be taken to indicate the actual distribution of AIB under experimental conditions. It is not central to the hypothesis to calculate the absolute concentrations of AIB in the various tissues, and this has not been done. Discussion is therefore in terms of the relative distribution of AIB between the tissues assayed.

The plasma levels of AIB-1-C^{14} acid of control and experimental animals are shown in Table I. The value of 1.62 for *t* is not significant with 15 degrees of freedom, indicating no real difference exists between the means. Tables 2 and 3 are similar to Table 1 and they deal with maternal liver and whole fetus tissues, respectively. In Table 2 the value of 3.21 for *t* indicates a significantly greater amount of AIB reached the livers of the experimental animals. There was, on the other hand, a significant decrease in the amount of AIB reaching the fetuses of the group fed 2,4,5-T, as indicated by the value for *t* of 2.20.

Table 1. AIB-1-C^{14} Acid in the Plasma of Pregnant Rats

Experimental Animal	Counts/ml	Controls Animal	Counts/ml
H1	9475	T1	7900
H2	11150	T2	9675
H3	8475	T3	10650
H4	8450	T4	13075
H5	9325	T6	14575
H6	10150	T7	11325
H7	11475	T8	9950
H8	7275	T9	10375
H9	10125	Mean	10940
Mean	9544	S.D.	1940
S.D.	1271		

$t = 1.62$, $p > 0.01$

Table 2. AIB-1-C^{14} Acid in the Liver of Pregnant Rats

Experimental Animal	Counts/gm	Controls Animal	Counts/gm
H1	73832	T1	32444
H2	85054	T2	48943
H3	87443	T3	59449
H4	132332	T4	63554
H5	94832	T6	67777
H6	85943	T7	62332
H7	64666	T8	52555
H8	68832	T9	54832
H9	52110	Mean	55235
Mean	82783	S.D.	10374
S.D.	21553		

$t = 3.21$, $p < 0.01$

Table 3. AIB-1-C^{14} Acid in Fourteen Day Rat Fetuses

Experimental Animal	Counts/gm	Controls Animal	Counts/gm
H1	6833	T1	6167
H2	5694	T2	6834
H3	4777	T3	7722
H4	4444	T4	8833
H5	7833	T6	5945
H6	6167	T7	8139
H7	5634	T8	6611
H8	6417	T9	8278
H9	7472	Mean	7316
Mean	6141	S.D.	999
S.D.	1074		

$t = 2.20$, $p < 0.05$.

Referring again to Table 1, it will be noted that there is a considerable degree of variation in the plasma levels of AIB-1-C^{14} acid across both groups. To account for the effect of this variation on the results of liver and fetal distribution, the data in Tables 1, 2 and 3 are displayed in graphical form in Figures 3 and 4. When counts per ml plasma are plotted against counts per gram liver, several patterns are obvious. The points on the graph for the control group fall in an easily distinguishable straight line. The points for the experimental group, however, appear to be randomly scattered above this line. When similar procedures are carried out to display the relationship between plasma and fetal levels of AIB-1-C^{14} acid, the results are less striking. Although several of the control points to lie in a linear relationship, one (representing animal T6) is clearly aberrant. The points for the experimental animals are again scattered. To quantitate these visual results, Pearson's correlation coefficient was calculated. Liver levels of AIB-1-C^{14} acid correlated significantly ($r = 0.89$) with plasma levels in the control group. With the point representing animal T6 removed, fetal levels of AIB-1-C^{14} correlated significantly ($r = 0.89$) with plasma levels in the control group. There was no significant correlation in either experimental group, even with high and low values thrown out.

2,4,5-T Feeding

The amount of food eaten by each animal in the two experimental groups and in a control group is recorded in Table 4. Group V, the control group which was force fed oil on days 9 through 14 of gesta-

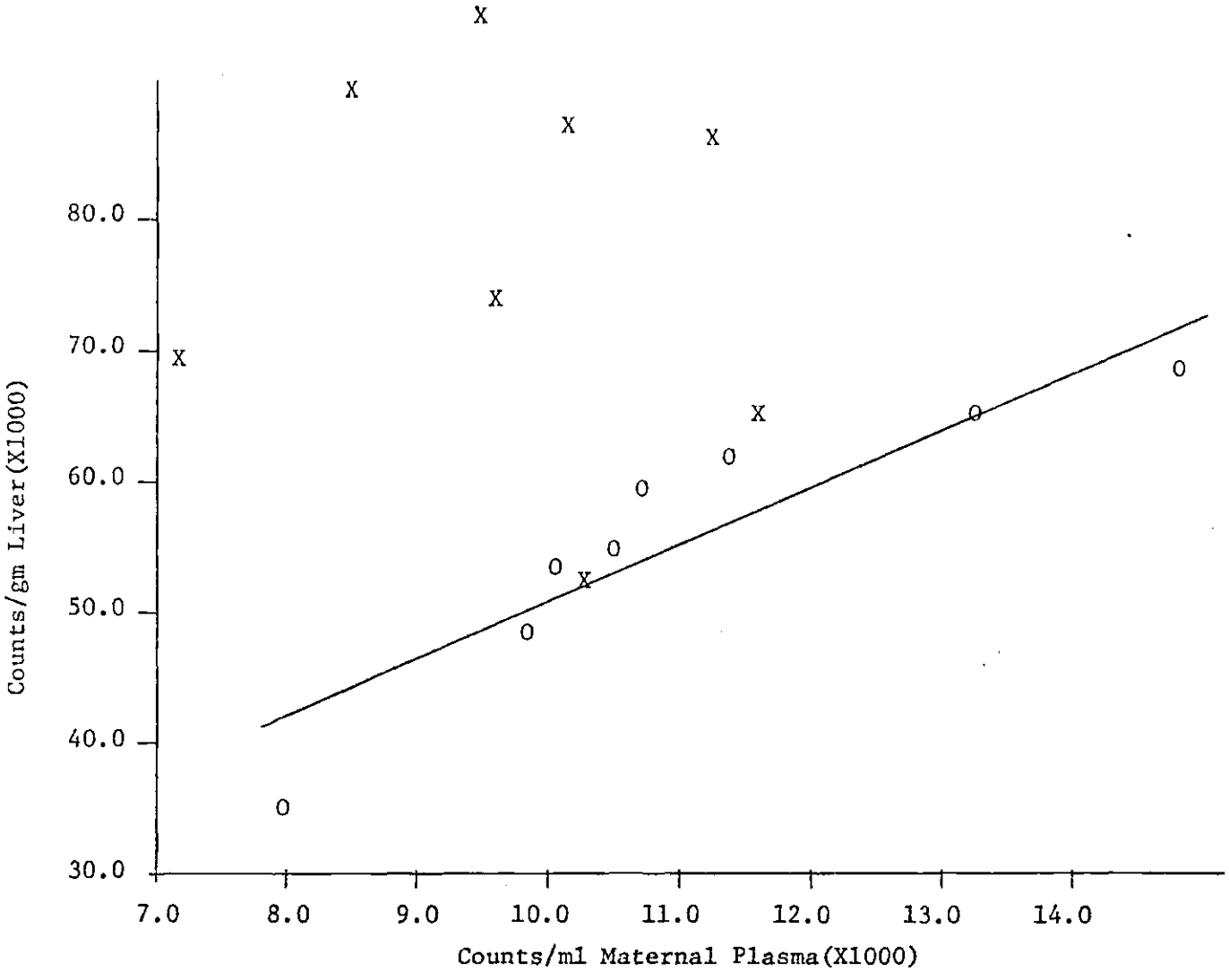

Figure 3. Correlation of Maternal Plasma and Liver AIB-1-C^{14} Acid

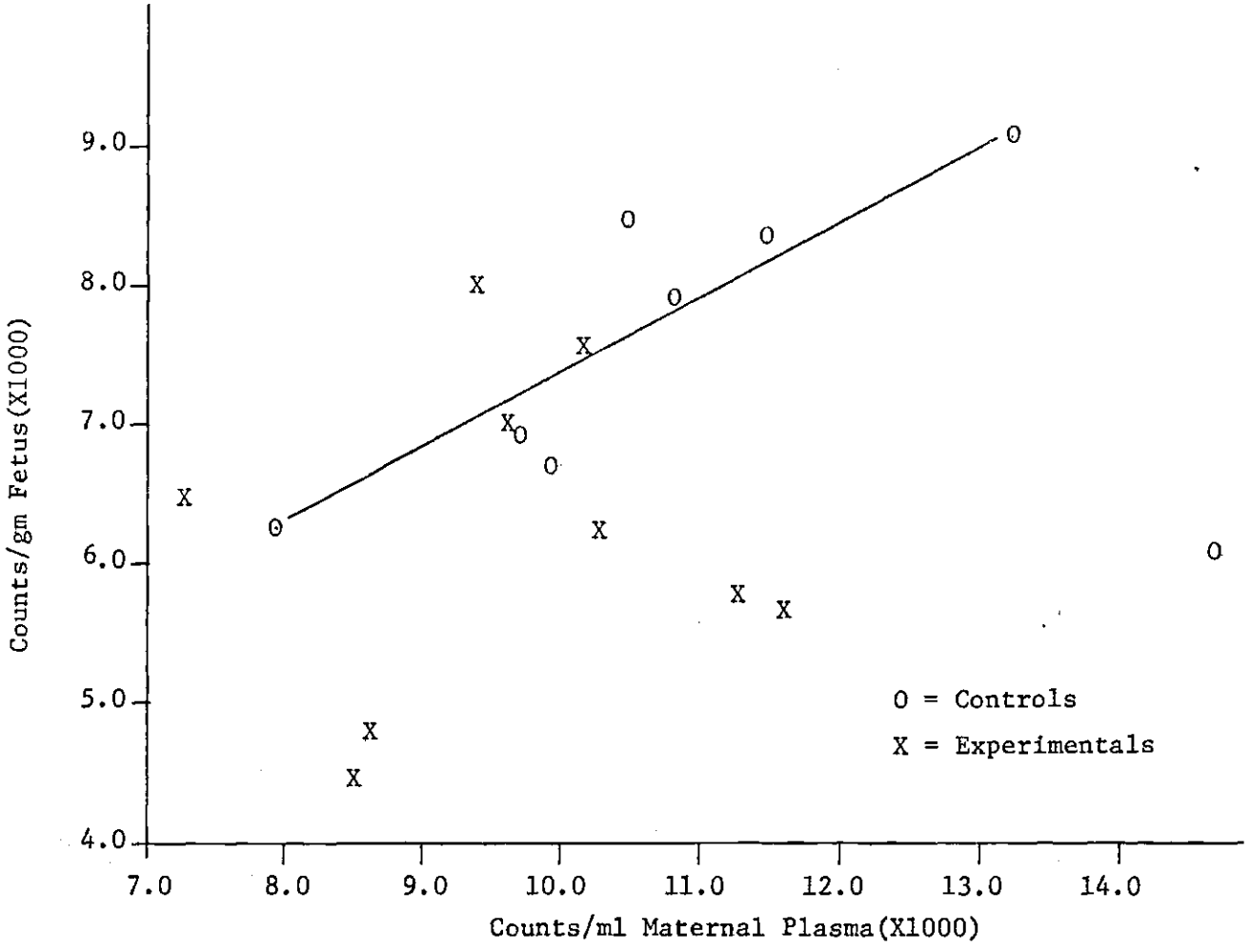

Figure 4. Correlation of Maternal Plasma and Fetal AIB-1-C^{14} Acid

tion, ate the most food per animal. Group I, the group which received 100 mg/kg of 2,4,5-T in oil on days 9 through 14, ate somewhat less, but the difference was not significant. Group III, the group which received 50 mg/kg of 2,4,5-T in oil on days 9 through 14, ate the least amount of food per animal. The difference between Group III and Group V was significant. There was no significant difference between the experimental groups.

The mean fetal weights for each litter in the experimental groups and their pair-fed controls are recorded in Tables 5 and 6. There was no significant difference between experimental animals and pair-fed controls according to Wilcoxon's signed rank test. Neither did the controls, consisting of the pair-fed Groups II and IV, the oil fed Group V and the untreated Group VI differ from each other when compared by the Mann-Whitman U-Test. The data for Groups V and VI are recorded in Table 7.

Table 4. Food Intake for Days 10-19 of Gestation

Experimental				Control	
Group I		Group III		Group V	
Animal	Amount (gm)	Animal	Amount	Animal	Amount
A	178	A	188	A	211
B	170	C	147	B	202
C	143	D	162	C	194
E	181	E	157	D	182
F	213	G	164	E	214
G	242	Mean	164	F	220
Mean	189	S.D.	13.5	G	218
S.D.	31.7			Mean	206
				S.D.	12.9

$-13.7 < X_I - X_{III} < 62.0$ (N.S.), $-52.1 < X_I - X_V < 17.5$ (N.S.)

$-78.1 < X_{III} - X_V < -4.9$*

*Significant at 5% level (Walker and Lev, 1969)

Table 5. Mean Fetal Weights of Groups I and II

Animal	Group I (100 mg/kg) Litter Size	Mean Wt.	Group II (Control) Litter Size	Mean Wt.
A	10	3.617 gm	11	3.811 gm
B	12	3.357	9	3.548
C	13	3.063	16	3.406
E	12	3.409	15	3.406
F	13	3.401	14	3.922
G	14	3.673	14	3.425

Table 6. Mean Fetal Weights of Groups III and IV

Animal	Group III (50 mg/kg) Litter Size	Mean Wt.	Group IV (Control) Litter Size	Mean Wt.
A	13	3.304 gm	11	4.323 gm
C	13	3.321	15	3.737
D	9	3.736	15	3.471
E	8	3.506	12	3.905
G	14	3.157	11	3.761

Table 7. Mean Fetal Weights of Groups V and VI

Animal	Group V (Control) Litter Size	Mean Wt.	Group VI (Untreated) Litter Size	Mean Wt.
A	11	3.666 gm	8	3.963 gm
B	14	3.467	9	4.049
C	11	3.867	10	3.639
D	9	3.995	17	3.623
E	14	3.767	17	3.631
F	12	3.973	16	3.894
G	12	3.757	-	-

CHAPTER IV

DISCUSSION

The degree of variability in plasma levels of AIB-1-C^{14} acid is greater than expected since the animals received entirely equivalent doses of compound and carrier. The doses were given in the same order that the animals had been fed 2,4,5-T, so there was minimal difference per animal in time of exposure to either drug. Whatever the source of the variation, it does not appear to be related to 2,4,5-T feeding.

The liver tissue of animals which were fed 2,4,5-T accumulated a significantly higher amount of AIB-1-C^{14} acid than that of controls. There are several possible explanations of this observation. The liver is the site at which metabolism of foreign compounds is carried out (Conney, A. H., 1967). The enzymes responsible for this matabolism are contained in the microsomes of the liver and have been shown to be inducible by various pesticides, drugs and other compounds (Conney, A. H., 1967). There was concern that the animals fed 2,4,5-T had experienced microsomal enzyme induction, which might account for an increase in the uptake of AIB-1-C^{14} acid by liver. However, it has not been shown that 2,4,5-T is capable of inducing microsomeal enzymes of the liver. Courtney (1970) injected rats with 2,4,5-T for five days and found only slight stimulatory effects on the metabolism of 2,4,5-T and no increase in the metabolism of pentobarbitol or phenylbutazone by liver slices. Since the animals in the study reported here

received 2,4,5-T on only one occasion, just 330 minutes before they were sacrificed, the possibility of microsomal enzyme induction accounting for the observed result lacks support.

A second plausible explanation of the increased uptake of AIB-1-C^{14} acid by liver of animals fed 2,4,5-T concerns the transport of amino acids from the plasma into the cells of the liver. Christensen (1965) has substantiated the existance of several transport systems for amino acids. The systems are somewhat specific for the amino acids they transport, but there is considerable overlapping. Characteristically, a high plasma level of a single amino acid will inhibit the transport of other amino acids which share the same system. Because of the overlapping of the systems, an imbalance in one may result in altered tissue levels of an amino acid considered to belong in another system. Christensen (1965) has said that the low specificity of the transport systems makes them more vulnerable than typical enzymic reactions to antagonisms among amino acids. It is conceivable that 2,4,5-T acting directly on these relatively nonspecific transport systems alters the normal balance of amino acids between the plasma and liver tissue. In this situation, however, AIB uptake by liver would be expected to be less than in controls.

Finally, the liver is responsible for removing amino acids from the plasma when gluconeogenesis is necessary. Glucagon is one of the hormonal messengers which instructs the liver to accumulate amino acids for this purpose. It has been shown that glucagon (Scott, et al., 1970) as well as epinephrine (Chambers, et al., 1972) in intact

animals, and hydrocortisone and insulin (Chambers, et al., 1965) in isloated liver cause increased AIB uptake by rat liver. If 2,4,5-T feeding solicits a response, due to physiological stress, for example, which involves the secretion of one or more of these hormones, it is possible that the observed increase in liver AIB uptake due to 2,4,5-T feeding is hormonally mediated.

There is a significant decrease in the amount of AIB-1-C^{14} acid reaching the fetuses of rats which were fed 2,4,5-T. The possibility that this reduction is due to the increased uptake by the liver of treated rats cannot be totally discounted since data was not recorded to allow calculation of the total pool of AIB-1-C^{14} acid do not exhibit a significant difference between control and experimental animals. Ccurtney (1970) and Fang, et al. (1973), have demonstrated the presence of 2,4,5-T at the placenta of rats in rather high levels. The results suggest that the reduction in the amount of the artificial amino acid crossing the placenta in 2,4,5-T fed animals is attributable to the biological activity of the herbicide in the rat. The results of this experiment do not give any indication of the exact nature of this activity, except to suggest that it may, because of the demonstrated presence of 2,4,5-T at the placenta, be more direct than in the case of liver.

The herbicide 2,4,5-T alters the relationship between plasma and tissue levels of AIB-1-C^{14} acid in the tissues analyzed. This observation is not inconsistent with the explanation that 2,4,5-T exerts an effect on the systems which transport amino acids from

plasma to tissue. Since it is an indirect effect on AIB-1-C^{14} acid transport, it is itself subject to the plasma and tissue levels of other amino acids. The effect of 2,4,5-T on AIB-1-C^{14} acid distribution may therefore vary from animal to animal.

One group of animals (Group III) fed 2,4,5-T consumed significantly less food than controls, while the other group (Group I) did not. It should be pointed out that the group eating the least food received the smaller dose of the herbicide. The fetal weights in this group were also the smallest of all the groups, although they did not differ significantly from controls. The observation that 50 mg/kg had a greater effect upon food intake and fetal weight than 100 mg/kg is surprising but in agreement with the results of other investigators (Neubert and Dillman, 1972) who have found no dose-response relationship in the reduction of fetal weight in 2,4,5-T fed mice. The results on food intake are based on a smaller than desirable number of animals.

CHAPTER V

CONCLUSION

The results of this experiment seem to support the hypothesis that 2,4,5-T influences the transport of AIB-1-C^{14} acid across the rat placenta. The hypothesis that 2,4,5-T feeding on days 9 through 14 of gestation reduces fetal weight is not supported. Observations in addition to those initially sought indicate that 2,4,5-T causes a significant increase in the uptake of AIB-1-C^{14} acid by the liver tissue of pregnant rats. Food intake was diminished for a small group of rats fed 2,4,5-T on days 9 through 14 of gestation.

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